Published in final edited form as:

Adv Immunol. 2013 ; 117: . doi:10.1016/B978-0-12-410524-9.00001-3.

Mechanisms of Epigenetic Regulation of Leukemia Onset and Progression

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Abstract

Over the past decade, it has become clear that both genetics and epigenetics play pivotal roles in cancer onset and progression. The importance of epigenetic regulation in proper maintenance of cellular state is highlighted by the frequent mutation of chromatin modulating factors across cancer subtypes. Identification of these mutations has created an interest in designing drugs that target enzymes involved in DNA methylation and posttranslational modification of histones. In this review, we discuss recurrent genetic alterations to epigenetic modulators in both myeloid and lymphoid leukemias. Furthermore, we review how these perturbations contribute to leukemogenesis and impact disease outcome and treatment efficacy. Finally, we discuss how the recent advances in our understanding of chromatin biology may impact treatment of leukemia.

Keywords

Leukemia; Epigenetics; Hematopoietic malignancies; Inhibitors; Drugs; Chromatin; Epigenetic modulators; DNA methylation

1. INTRODUCTION

1.1. Leukemia as a heterogeneous and multifactorial disease

Hematopoietic malignancies are a broad category of diseases (Gilliland, 2001). Leukemia is one of the most aggressive among them and is characterized as the abnormal proliferation of immature cells of the hematopoietic system. Different types of leukemias can arise from lymphocytes (lymphocytic leukemia), myeloid cells (myeloid leukemia), erythrocytes (erythrocytic leukemia), and others in the bone marrow, lymph nodes, or spleen. Regardless of the cell type of origin, leukemia generally proceeds in either a chronic or an acute manner. Chronic disease consists of a long incubation period, whereas acute leukemia is associated with an abrupt accumulation of immature blood cells in the peripheral blood, bone marrow, and secondary lymphoid organs. Certain disorders are marked by both a chronic and acute phases, which are categorized based on several factors. Among the most

common forms of leukemia are two chronic variants, chronic myeloid leukemia (CML) and chronic lymphoblastic leukemia (CLL), and two acute variants, acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). We briefly review these disease types below.

1.1.1 Chronic myeloid leukemia—CML is a unique case of leukemia that is characterized by the presence of the Philadelphia chromosome. This reciprocal translocation between chromosomes 9 and 22 leads to the formation of a chimeric protein consisting of the breakpoint cluster region (*BCR*) gene with the abelson kinase (*ABL*) gene. The resulting Bcr-Abl oncogene is characterized by constitutive tyrosine kinase activity leading to activation of downstream targets (Bartram et al., 1983; Druker, 2008). The current standard of care for CML is the small-molecule kinase inhibitor Imatinib, a very specific inhibitor of the Bcr-Abl fusion protein. Treatment with Imatinib results in a 5-year progression free survival rate of approximately 89% (Druker et al., 2006). Resistance to Imatinib occurs in certain cases usually through mutations in the Imatinib binding site in Bcr-Abl (Deininger, Goldman, & Melo, 2000; Holtz, Forman, & Bhatia, 2005). It is also worth noting that Imatinib does not eradicate the disease, as it apparently does not target the CML leukemia-initiating cells (LICs).

1.1.2 Acute myeloid leukemia—AML is the most common acute leukemia and its incidence increases with age (Daver & Cortes, 2012). AML can either occur *de novo* or be preceded by a premalignant state. Several preleukemic conditions exist (Byrd et al., 2002) which have the potential to progress to AML. Myelodysplastic syndromes (MDS) or -myeloproliferative neoplasms (MPN) are characterized by a block in differentiation leading to accumulation of myeloid progenitor cells. Included in MDS and MPN are refractory anemia (RA), chronic myelomonocytic leukemia (CMML), polycythemia vera (PV), essential thrombocytosis (ET), and myelofibrosis (MF). Around one-third of the MDS cases progresses and gives rise to AML.

AML is a heterogeneous disease that can be classified in as many as seven subtypes (de Jonge, Huls, & de Bont, 2011). These subtypes are characterized by a variety of cytogenetic and cell surface markers. Unlike CML, there is no unifying way of treating AML patients. In general, AML is treated with an array of chemotherapeutic drugs; in some cases, chemotherapy is followed by bone marrow transplantation. Overall, AML can be very hard to treat, resulting in a relatively high mortality, which is reported to account approximately to 10,000 deaths per year in the United States.

1.1.3 Acute lymphoblastic leukemia—ALL is an acute disorder of either B-lymphocytes (B-ALL) or T-lymphocytes (T-ALL). ALL is the most common form of cancer in children (Pui & Evans, 2006). The genetics of ALL are quite complex and are comprised of a variety of chromosome fusions. Similar to AML, these chromosome fusions can be used to distinguish different subtypes of disease, which are associated with distinct clinical features and outcome.

In T-ALL, the most common genetic event is the activation of the Notch pathway. Mutations leading to enhanced Notch signaling are present in more than 50% of patients (Aifantis, Raetz, & Buonamici, 2008). This can be explained by the fact that in thymic development, signaling through the Notch receptor promotes cell cycle progression and proliferation and Notch1 therefore acts as a proto-oncogene in this setting.

Treatment of ALL especially in children has become very effective, leading to cure rates as high as 80% (Chessells et al., 2003; Rivera et al., 2005). This is mainly achieved by the use

of advanced chemotherapy regimen. Although this is an outstanding clinical achievement, novel less toxic treatments should still be pursued.

1.1.4 Chronic lymphocytic leukemia—Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia (Cramer & Hallek, 2012). CLL is a disease of the B-cell lymphocytes that is characterized by a very slow progression. The incidence of CLL increases with aging. Progression of the disease can be at such a low rate that treatment is sometimes postponed till later stage. As in the leukemias described above, chromosomal aberrations and gene mutations (including mutations in the NOTCH pathway) are common in CLL. And, again, these genetic variants also determine disease outcome.

1.2. Epigenetic factors and their possible roles in leukemia

The focus of this review is the regulation and deregulation of epigenetic processes in different types of leukemia. The term epigenetics was coined by C.H. Waddington in the 1940s and is a fusion of words “genetics” and “epigenesis.” The major meaning of epigenesis at that time was that the embryo gradually changes into the adult organism in contrast to the prevailing idea of that era that the adult is preformed at the embryo stage. A more modern definition of epigenetics has been proposed as “a change in the state of expression of a gene that does not involve a mutation, but that is nevertheless inherited in the absence of the signal (or event) that initiated the change” (Ptashne, 2007). The term is used for phenomena such as genomic imprinting, paramutation, polycomb complex-mediated gene silencing, and position effect variegation. Model organisms have proven to be incredible tools to obtain insight in epigenetic phenomena. For instance, *Drosophila* development allows the study of stem cells that are responsible for the formation of adult structures in the fly.

Epigenetic phenomena must fit at least one of the following three criteria (Bonasio, Tu, & Reinberg, 2010): (i) a mechanism for propagation, the signal must be propagated through DNA replication/cell division; (ii) the signal must be transmitted to the progeny; and (iii) the signal should affect gene expression. Among, the modifications that fit these criteria are histone modifications, histone variants, DNA methylation, relative nucleosomal position, and occupancy and larger chromatin domains (Margueron & Reinberg, 2010). To date, DNA methylation is the only epigenetic mark that fulfills all three criteria. Many different mechanisms have been proposed that would explain the propagation and transmission of histone marks, or the histone variants; however, these processes are not completely understood currently and require further investigation (Bonasio et al., 2010).

Recently, it has become clear that disruption of epigenetic processes contributes to leukemic transformation. Traditionally, mutations in leukemia were thought to involve two discreet classes of genes. One class contains genes whose mutation can give a proliferation or survival advantage to the cell and is not specific to the hematopoietic system. This would include components of RAS-MAPK signaling, PI3-kinase/AKT signaling, and others. A second class of genes mutated in leukemia consists of regulators of hematopoiesis which do not necessarily give a growth or survival advantage but result in differentiation defects (Gilliland, 2001; Shih, Abdel-Wahab, Patel, & Levine, 2012). Although epigenetic regulators often do not belong to either of these two classes of genes, they are nonetheless frequently mutated in leukemia. This is exemplified by the translocations that are commonly found in leukemia and affect mixed lineage leukemia (*MLL*), polycomb repressive complex 2 (*PRC2*), or the ten-eleven translocation (*TET*) family. It has been proposed that deregulation of epigenetic factors can provide a tumor cell the plasticity needed to adapt to different situations. Similarly, it is thought that perturbation of epigenetic regulators prior to full transformation may be a priming event that allows a more permissive environment for

leukemogenesis upon acquisition of additional mutations (Feinberg, 2007). Apart from the enzymes that catalyze the histone or DNA modifications (epigenetic writers), there are proteins that specifically bind modified histone residues (readers), as well as enzymes that remove covalent modifications (erasers). There are enzymes containing the appropriate domains for both reading and writing of the marks. Mutations that alter enzymatic function can be found in all these types of chromatin-interacting proteins.

In this review, we will discuss the major perturbations to epigenetic processes found in leukemia. For purposes of clarity, we will divide this review into three sections comprising the following: (1) DNA methylation; (2) polycomb and MLL complexes and their roles in physiology and disease; and (3) other epigenetic modulators, with an emphasis on the ones that are mutated in leukemia. Moreover, novel therapeutic options will be mentioned throughout the review, such as inhibitors of epigenetic modulators and their combinations with current therapies. Finally, emerging technologies and biological paradigms and how the potential for novel-targeted therapies will be discussed. Of course, the field is enormous and this review cannot cover every aspect of epigenetic regulation in leukemia; we thus apologize to our colleagues for any potential omissions.

2. ABERRANT DNA METHYLATION IN LEUKEMIA

2.1. The role of DNA methylation in hematopoietic malignancies

DNA methylation is the most common epigenetic modification. Methylation of CpG islands in the promoters of genes is generally associated with reduced expression from that locus. CpG islands can be at least 200 bases in size with a GC content of at least 50%. CpG dinucleotides are quite rare in mammalian genomes (~1%) (Esteller, 2008), despite which about 60% of human promoters contain CpG islands. Although the majority of CpG islands are unmethylated, a small percentage (~6%) becomes methylated in a tissue-specific manner during early development or in differentiated tissues (Straussman et al., 2009). Besides CpG island methylation in the promoter, DNA methylation of the gene body is common. This is mainly seen in ubiquitously expressed genes and is positively correlated with gene expression (Hellman & Chess, 2007). It has been proposed that gene body DNA methylation might increase elongation efficiency and prevent spurious initiation of transcription (Zilberman, Gehring, Tran, Ballinger, & Henikoff, 2007).

Aberrant methylation patterns are considered to be one of the characteristics of the cancer epigenome (Laird & Jaenisch, 1996). In general, global DNA hypomethylation is observed which can lead to chromosomal instability (Eden, Gaudet, Waghmare, & Jaenisch, 2003; Gaudet et al., 2003; Holm et al., 2005; Nishigaki et al., 2005). This general hypomethylation can lead to aberrant activation of oncogenes such as cyclin D2 and maspin (Oshimo et al., 2003). On the other hand, hypermethylation of the promoters of tumor-suppressor genes such as retinoblastoma 1, *CDKN2A* (also known as cyclin-dependent kinase inhibitor p16), the von Hippel–Lindau tumor suppressor, and MutL protein homologue 1 can lead to aberrant silencing (Esteller, 2007; Herman & Baylin, 2003; Jones & Baylin, 2002). Therefore, it is not surprising that DNA methyltransferase (DNMT) enzymes, which catalyze the addition of a methyl group to CpG dinucleotides, play key roles in development and disease. DNMT1 is considered to be the maintenance methyltransferase and can act on unmethylated DNA. DNMT3A and DNMT3B are the *de novo* DNMTs, whereas DNMT3-like lacks catalytic activity but acts as cofactor for DNMT3A/B and interacts and colocalizes with them in the nucleus.

Only recently, it became clear that mutations in DNMT3A are common in AML (Ley et al., 2010; Yan et al., 2011). Moreover, there has been an advent of specific DNMT inhibitors that are used against MDS with very encouraging results (Dawson & Kouzarides, 2012).

2.2. The role of DNMT3A in leukemia

2.2.1 DNMT3A mutations in hematopoietic malignancies—Mutations in *DNMT3A* (Fig. 1.1) were reported in approximately 20% of cases of AML of various subtypes (Ley et al., 2010). Identical mutation percentages were found in the AML-M5 subtype that is classified as acute monocytic leukemia (Yan et al., 2011). In addition, it was reported that DNMT3A is mutated in other hematopoietic malignancies albeit at a lower frequency (Thol et al., 2011; Walter et al., 2011). *DNMT3A* mutations seem not to be restricted to leukemias from the myeloid lineage, as recently mutations have also been found in T-cell lymphoma and T-ALL (Couronne, Bastard, & Bernard, 2012; Simon et al., 2012). In pediatric AML, however, mutations in DNMT3A have not been found, despite the sequencing and analysis of a cohort consisting of 180 patients (Ho et al., 2011).

2.2.2 Functional consequence of DNMT3A mutations—So far, mutations identified in *DNMT3A* are found to be exclusively heterozygous. Specifically, a very clear hotspot can be identified for *DNMT3A* mutations, as around 50% of the mutations occur in residue R882 (Ley et al., 2010). *In vitro* experiments showed that AML-linked mutations in DNMT3A lead to a severe loss of enzymatic activity. However, as DNMT3A is one of two *de novo* DNMTs in the human genome, it is unclear what the molecular consequence of DNMT3A mutations is. One study compared the DNA methylation status of *DNMT3A* mutant versus wild-type AML samples. This revealed that, as expected, some CpG islands in promoters indeed become hypomethylated. In AML samples with mutant DNMT3A, a lower level of methylation of CpG islands in the HoxA-gene cluster was detected. In addition, there was a clear correlation with increased expression of the HoxA genes, which leads to a less differentiated phenotype (Yan et al., 2011).

2.2.3 Mouse models of DNMT3 function—Initially, no apparent hematopoietic stem cell (HSC) differentiation defect was observed in mice mutant for either *DNMT3A* or *DNMT3B* (Tadokoro, Ema, Okano, Li, & Nakauchi, 2007). It was found that cells deficient for *DNMT3A* or *DNMT3B* could still give rise to a variety of progenitors. In addition, it was shown that HSCs depleted for both DNMT3A and DNMT3B could not reconstitute hematopoiesis of a recipient animal. After the identification of the *DNMT3A* mutations in AML, Challen et al. (2012) further examined the *DNMT3A* knockout phenotype. In this case, it was found that loss of *DNMT3A* led to decreased differentiation of mouse HSCs. This phenotype could be correlated with higher expression of genes that are involved in maintaining multipotency of HSCs. Strikingly, when comparing methylation patterns in wild type and *DNMT3A* mutant cells, no significant changes were found in overall DNA methylation. However, further analysis of specific loci revealed that some genes were hypomethylated while others were hypermethylated in *DNMT3A* knockout animals. Genes that were found to be hypomethylated and consequently higher expressed include the well-known HSC homeostasis genes *RUNX1* and *GATA3*.

2.2.4 Is mutant DNMT3A a prognostic marker in myeloid leukemia?—The genetics of AML are very complex, but, nevertheless, it has been reported that the mutation status of *DNMT3A* by itself is a significant prognostic marker for disease outcome in AML (Ley et al., 2010; Marcucci et al., 2012; Ribeiro et al., 2012). Common genetic lesions co-occurring with mutant DNMT3A are mutations in *NPM1* and *FLT3*. Especially, the combination of an FLT3-ITD mutation combined with mutant *DNMT3A* seems to be associated with unfavorable outcome in this disease (Patel et al., 2012). Moreover, one report showed that patients with *DNMT3A* mutations could benefit from higher than normal dose of chemotherapy (Daunorubicin) (Patel et al., 2012).

2.2.5 DNMT inhibitors—Currently, two DNMT inhibitors, vidaza (5-azacytidine) and decitabine (5-aza-2-deoxycytidine), are approved for the treatment of cancer patients. Both vidaza and decitabine are analogues of the nucleotide cytosine. The relatively low side effects make these DNMT inhibitors the drug of choice for the treatment of MDS. However, in the more advanced AML, the use of decitabine is debated and is reported to have very little effect. Nevertheless, combination of inhibitors for histone deacetylases (HDACs) with DNMTi has given even more very promising outputs (Gore, 2011).

Another preliminary study used a small cohort of patients in which it seems that *DNMT3A* mutant AMLs are more sensitive to the DNA methylation inhibitor Decitabine (Metzeler et al., 2012). The mechanism behind these responses is currently unknown.

2.3. The biology of TET proteins and their perturbations in leukemia

2.3.1 TET proteins—In a search for proteins that are homologous to the J-binding proteins from the parasite leishmania, the only homologous proteins identified in the human genome were the TET proteins (Tahiliani et al., 2009). J-binding proteins were known for their capacity to bind a modified DNA base, base J that is unique for the parasite. Base J is a glycosylated derivative of the base thymidine. This suggested a role for the TET proteins in modifying DNA directly. Indeed, further studies showed that in TET proteins resides the catalytic activity to modify 5-methylcytosine to 5-hydroxymethylcytosine (5-hmC) (Tahiliani et al., 2009).

Soon after this finding, Delhommeau et al. (2009) reported frequent mutations of *TET2* in AML again suggesting a key role for DNA methylation in leukemogenesis. Some of these mutations were later verified to be true loss-of-function variants (Ko et al., 2010); however, the role of 5-hmC in tumor development remains to be fully appraised. A variety of technical issues have hampered the study of TET2 and 5-hmC in leukemia. The lack of a TET2 antibody, for instance, has made it difficult to study its genomic occupancy. However, recent technical advances have made genome-wide 5-hmC profiling possible with base-pair resolution (Booth et al., 2012; Yu et al., 2012).

2.3.2 Mutational status of TET proteins in leukemia—First identified as a gene (*TET1*) in a chromosomal translocation in AML, it took some time to appreciate the importance of the TET proteins in leukemia. The initial report described a fusion between chromosomes 10 and 11 in AML that lead to a chimeric protein consisting of the MLL amino(N)-terminus and the carboxyl(C)-terminus of *TET1* (Lorsbach et al., 2003). In addition, despite the fact that fusions and deletions in chromosome 4 were detected in a number of AML patients (Viguie et al., 2005), it was only later understood that a single gene was present in this locus. The gene product showed a high level of sequence conservation with the previously identified TET1 protein and was therefore named TET2. Further analysis of the human genome identified one more protein homologous to both TET1 and TET2, that is, TET3 (Delhommeau et al., 2009). Fusions of *TET1* and deletions of the *TET2* locus indicated an important role of the TET proteins in hematopoietic malignancies. And, indeed, sequencing efforts confirmed that *TET2* (Fig. 1.1) is a commonly mutated gene in myeloid leukemia and premalignant stages of leukemia. So far, *TET2* mutations have been found in AML, CMML, MDS, and other myeloid malignancies. The largest studies suggest that *TET2* mutations can be identified in 2–10% of PV and ET patients, and in 10–20% of patients with primary MF or post-PV/ET MF (Abdel-Wahab et al., 2010, 2009; Cimmino, Abdel-Wahab, Levine, & Aifantis, 2011; Tefferi et al., 2009). In addition, studies of paired MPN and AML samples from individual patients demonstrated that *TET2* mutations are commonly acquired during transformation to AML from a chronic myeloid neoplasm (Abdel-Wahab et al., 2010). Surprisingly, mutational analysis of both *TET1* and

TET3 has not been as fruitful. Mutations in *TET1* and *TET3* have been reported in patients with CLL, but the overall incidence of these mutations is currently unknown (Quesada et al., 2012). One of the few studies that carefully investigated the status of all the three TET family members in myeloid malignancies found only *TET2* and not *TET1* nor *TET3* mutated (Abdel-Wahab et al., 2009). Recently, *TET2* has been found to be also mutated in lymphoid neoplasms (Couronne et al., 2012; Quivoron et al., 2011). Finally, recent studies point to a role for TET proteins in solid tumors, as sporadic mutations have been identified in brain (Parsons et al., 2011) and prostate cancers (Grasso et al., 2012).

2.3.3 Consequence of TET2 mutations in AML—The majority of our knowledge of the TET proteins comes from studies performed in nonhematopoietic cells. For instance, one study that sheds light on how TETs could be involved in active DNA methylation was performed in the brain (Guo, Su, Zhong, Ming, & Song, 2011). The proposed mechanism involves activation-induced (cytidine) deaminase (AID) and APOBEC proteins, which promote the conversion of 5-hmC into an unmodified cytosine and thereby lead to active demethylation of the DNA. If this mechanism were proven to be universal, the consequence of loss of *TET2* function would be increased DNA methylation. This does pose some sort of a conundrum as we have discussed earlier that loss-of-function mutations in *DNMT3A* are common in AML. So far, however, it is not clear what happens on the level of DNA methylation in *TET2* mutant cells. There is conflicting evidence in the literature reporting that *TET2* loss could lead to either increased or decreased DNA methylation (Figueroa et al., 2010; Ko et al., 2010).

2.3.4 TET2 mouse models—Several research groups, including ours, have modeled the loss of *TET2* in the hematopoietic system (Li et al., 2011; Moran-Crusio et al., 2011; Quivoron et al., 2011). In all cases, the loss of *TET2* leads to a decrease in 5-hmC levels as expected. Deletion of *TET2* in the HSC compartment causes an increase in self-renewal capacity. During the maturation of the *TET2* knockout animals, an increase in the frequency of both myeloid and lymphoid cells can be observed. This premalignant state develops into a myeloproliferative neoplasm as the mice become older; this results in splenomegaly and either an MDS or a CMML-like disease.

2.4. IDH1 and IDH2 oncometabolic proteins

2.4.1 IDH1 and IDH2 mutations in leukemia—The isocitrate dehydrogenase (IDH) enzymes are NADP-dependent molecules that normally function as homodimers to catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (a-KG) with the concomitant production of NADPH. Mutations in *IDH1* and *IDH2* are important for our discussion for two reasons. First, the mutations occur in a hotspot resulting in the alteration of the enzymatic activity of the enzyme. Second, inhibition of *TET2* seems to be part of the mechanism by which mutations in *IDH1* and *IDH2* cause leukemia (Figueroa et al., 2010). The first indication that the IDH enzymes were involved in carcinogenesis came from a study in gliomas (Yan et al., 2009). Strikingly, it was found that nearly all mutations occur in a couple of residues in either *IDH1* (R132) or *IDH2* (R140 or R172) (Fig. 1.1). Not much later mutations in *IDH1* and *IDH2* were detected in hematopoietic malignancies. Especially, myeloid malignancies are reported to have mutations in either *IDH1* or *IDH2* at a rate of around 9% (Green & Beer, 2010; Ward et al., 2010). In particular, mutations have been reported in MDS and MPN (Kosmider et al., 2010).

2.4.2 Consequence of IDH mutations—As mentioned above, mutations in the *IDH1* and *IDH2* proteins occur in the protein catalytic site. Wild-type IDH enzymes can convert isocitrate to a-KG. *IDH1* is predominantly cytoplasmic, while *IDH2* can be found in the cell mitochondria. So far, only heterozygous *IDH* mutations have been found, leaving one allele

intact. One very exciting finding was the fact that mutations in IDH proteins do not abrogate its enzymatic function but change the outcome of its reaction toward isocitrate. Mutant IDH proteins have a much higher output of 2-hydroxyglutarate (2-HG) at the expense of α -KG (Ward et al., 2010). 2-HG is an oncometabolite that can be used as a marker to distinguish wild-type *IDH* from mutant *IDH* cancers. At the molecular level, this also somehow explains how mutations in an enzyme, so critical for cellular homeostasis, can be tolerated. Mutations in *IDH1* and *IDH2* seem to have similar effects on the enzyme function. It is therefore not surprising that mutations in *IDH1* and *IDH2* are mutually exclusive.

Recent studies showed a role for oncometabolites, such as 2-HG, in the function of epigenetic modulators (Teperino, Schoonjans, & Auwerx, 2010). Under normal conditions, α -KG is produced in the trichloroacetic acid (TCA) cycle from isocitrate and is a cofactor for dioxygenases. Among these dioxygenases are the Jumonji-domain-containing histone demethylases, as well as the Tet family of hydroxymethylases. As we have discussed earlier, mutations in *IDH1* and *IDH2* lead to the increased production of 2-HG, leading to reduced catalytic activity of certain dioxygenase enzymes. In an elegant study, this hypothesis was proven (Figueroa et al., 2010). First, it was shown that DNA isolated from *IDH* mutant AMLs is more hypermethylated. Second, it was shown that mutations in IDH proteins inhibit the conversion of 5-mC into 5-hmC by TET proteins. This observation is supported by the fact that *TET2* and *IDH* mutations are mutually exclusive in AML (Figueroa et al., 2010). Other enzymes that are affected by IDH mutations are the histone demethylases, especially the H3K9me3 demethylase KDM4C (Lu & Thompson, 2012; Lu et al., 2012; Turcan et al., 2012).

Along these lines, it is possible that mutations of other enzymes in the TCA cycle can cause similar effects. Examples of these are *loss-of-function* mutations in the enzymes succinate dehydrogenase and fumarate hydratase (Kaelin, 2011).

2.4.3 Animal models for IDH gene function—Very recently, the *IDH1* mutation commonly found in AML (R132H) was modeled in a mouse (Figueroa et al., 2010). This mutation was created in a conditional fashion in one of the endogenous *IDH1* alleles. This murine model showed that mutant *IDH1* is indeed sufficient to disrupt the hematopoietic system homeostasis. *IDH1* mutant mice show extra medullary hematopoiesis and a loss of cells from the bone marrow. This phenotype is associated with an increase in methylation of both DNA and histones.

3. DISRUPTION OF HISTONE-MODIFYING COMPLEXES POLYCOMB AND MLL IN LEUKEMIA

Posttranslational modification of N-terminal histone tails is a more recently appreciated mechanism of regulation of gene expression patterns in development and disease. Since Jenuwein and Allis (2001) first proposed the “histone code” hypothesis in 2001, there has been an explosion in research aimed at cataloging all posttranslational modifications added to the histones and their distribution across genomes as well as association with particular transcriptional states (Ernst et al., 2011; Tan, Luo, et al., 2011). Additionally, many groups have focused on understanding how enzymes that catalyze or remove these modifications and other proteins with the ability to “read” histone marks are involved in global regulation of chromatin states (Shih et al., 2012; Zhou, Goren, & Bernstein, 2011). The importance of such enzymes in disease development is highlighted by frequent mutation of many key histone modifiers in human cancer (Abdel-Wahab et al., 2012; Dawson, Kouzarides, & Huntly, 2012; Ntziachristos et al., 2012; Patel et al., 2012; Shih et al., 2012; van Haaften et al., 2009; Zhang, Ding, et al., 2012), including both solid tumors and hematological neoplasms. Deregulation of mechanisms regulating histone modification seems to have a

particularly important role in leukemic transformation as genetic lesions targeting such proteins are often considered driver mutations, with potent oncogenic activity. Here, we will focus on two histone-modifying complexes, the PRC, including both PRC2 and PRC1, and MLL complexes, which are frequently perturbed in human leukemia of several different blood lineages.

3.1. PRC2 in hematological neoplasms

PRC2 is a large multimeric enzymatic complex that includes the set-domain-containing methyltransferase EZH2. Other key components include chromodomain-containing protein EED, SUZ12, and histone chaperone RBBP4/7 (Margueron & Reinberg, 2011) (Fig. 1.2A). As its name suggests the main function of this complex is to silence gene expression at specific loci through catalysis of trimethylation of lysine 27 of histone 3 (H3K27me3). The presence of this mark not only enhances the activity of PRC2 itself but is also read by the polycomb repressive complex 1 (PRC1), leading to monoubiquitylation of histone 2A lysine 119 and subsequent chromatin compaction (Simon & Kingston, 2009). Gene silencing by PRC2 is critical for establishing proper lineage commitment during development by inactivating genes required for alternative cell fates. With a critical role in nearly every developmental system, it is not surprising that deregulation of PRC2 function contributes to tumorigenesis (Bracken & Helin, 2009; Margueron & Reinberg, 2011; Sauvageau & Sauvageau, 2010; Sawarkar & Paro, 2010).

Although components of PRC2 are heavily mutated in many types of cancer, the consequences of such mutations in leukemia are especially intriguing with reports of both oncogenic and tumor-suppressor function of the complex in neoplasms derived from different lineages (Ernst et al., 2010; Morin et al., 2010) (Fig. 1.2B). Originally suggested to be a *loss-of-function* mutation, recurrent mutations in *EZH2* residue Y641 have now been shown to enhance PRC2 activity by cooperating with complexes containing the wild-type EZH2 protein leading to more efficient catalysis and hypertrimethylation of lysine 27 of histone 3 (H3K27me3) in follicular and diffuse large B-cell lymphoma (DLBCL) (McCabe et al., 2012; Sneeringer et al., 2010; Yap et al., 2011). Conversely, in T-cell acute lymphoblastic leukemia (T-ALL), *loss-of-function* mutations to several PRC2 subunits including EZH2, SUZ12, and EED have been reported to result in a more aggressive phenotype compared to wild-type tumors (Ntziachristos et al., 2012; Simon et al., 2012), suggesting a tumor-suppressor role for the complex in this context. Unlike DLBCL, T-ALL mutations targeting PRC2 components consist mainly of nonsense mutations upstream of the catalytic domain of EZH2 and larger deletions of the locus, suggesting a true loss-of-function outcome. Further highlighting the duality of PRC2 function in hematological tumors, it has been suggested that within different subtypes of myeloid disease both an oncogenic and a tumor-suppressor function for this complex exist. Ernst et al. (2010) have shown loss-of-function *EZH2* mutations in MDS and MPN with poorer overall survival in patients with mutant alleles. However, in mouse models of MLL-AF9 positive AML, it seems that PRC2 is required for efficient transformation, suggesting a role for the complex in contributing to aberrant self-renewal of LICs (Neff et al., 2012; Shi et al., 2012). These results suggest that proper maintenance of the H3K27me3 modification is critical for normal cell homeostasis.

Although the results discussed above are compelling, our understanding of the mechanism through which deregulated H3K27me3 might lead to leukemic transformation is very poorly understood. As a tumor suppressor, we might imagine an antagonistic relationship between PRC2 and oncogenic transcription factor networks. In diseases driven by transcriptional activators, we support a model where genes targeted for activation by the oncogenic factor might in turn be occupied for silencing by PRC2. Thus, loss of PRC2 function may create a more permissive environment for the activity of oncogenic transcription factors. As an

oncogene, there is evidence that PRC2 can act to directly repress key tumor-suppressor genes such as the *CDKN1A*, *CDKN1B* (Velichutina et al., 2010), or *CDKN2A/CDKN2B* (Chen et al., 2009) loci providing a mechanism of epigenetic silencing in lieu of genetic inactivation.

Recently, mutations of the protein ASXL1, that is part of the polycomb-repressive deubiquitylase complex, were identified in human malignancies (Abdel-Wahab et al., 2012; Shih et al., 2012). This complex catalyzes the deubiquitination of H2AK119, the modification left by PRC1, suggesting a possible antagonistic relationship. Surprisingly, inactivation of ASXL1 has been shown to have a potent effect on PRC2 function leading to global decreases in H3K27me3 although the mechanism by which this occurs is unclear. However, loss of ASXL1 and PRC2 function at the *HOXA* cluster was shown to correlate with increased *HOXA9* expression which is known to contribute to myeloid transformation (Abdel-Wahab et al., 2012).

3.2. Role of PRC1 in leukemia

Like PRC2, PRC1 has also been suggested to play a role both in maintenance of HSCs and transformation *in vivo*. However, unlike PRC2, there are very few reports of mutations in PRC1 complex members in cancer. Specifically, there are studies showing that PRC1 component *BMII* is required for normal HSC function and similarly for maintenance of leukemic stem cell function in MLL-rearranged leukemia (Oguro et al., 2012; Park et al., 2003). It has been proposed in both settings that *BMII* is essential for maintaining PRC1-mediated suppression of the *CDKN2A/CDKN2B* locus, thus allowing cells to evade cellular senescence. Nevertheless, mutations in *BMII* have not been described so far.

3.2.1 Histone methyltransferase inhibitors—Chaetocin, deazaneplanocin (DZNep), and BIX-01294 are the best characterized histone methyltransferase inhibitors. All these inhibitors have so far only been tested in the preclinical environment. However, at this stage, results are promising; for example, chaetocin has anticancer properties against multiple myeloma (MM) cells (Greiner, Bonaldi, Eskeland, Roemer, & Imhof, 2005; Isham et al., 2007). Combination of the PRC2 (EZH2) inhibitor DZNep and a HDAC inhibitor (HDACi) (Panobinostat) has been shown to kill AML cells *in vitro* (Fiskus et al., 2009).

3.3. MLL function

The MLL gene is the human homologue of *Drosophila melanogaster* trithorax. Trithorax was initially described as a regulator of homeotic gene expression in flies. Now, it has become clear that MLL is a key component of mammalian COMPASS-like complexes, which play critical roles in both embryonic development and hematopoiesis. COMPASS complexes contain hSET1A and B, MLL1, MLL2, MLL3, or MLL4 as the catalytic subunit and have a critical role in activating transcription by catalyzing mono-, di-, and trimethylation on lysine 4 of histone 3 (H3K3me1, H3K4me2, H3K4me3). WDR5, RBBP5, and ASH2L are important core subunits that modulate the action of the methyltransferase (Dou et al., 2006). What determines the catalytic specificity of the complex regarding the component constitution is still unknown as deletion of any of the four catalytic subunits leads to minimal effects in the H3K4me3 levels possibly because of redundancy. However, deletion of the core subunits brings about global loss of H3K4me3 (Lubitz, Glaser, Schaft, Stewart, & Anastassiadis, 2007; Wang, Lin, et al., 2009). In this regard, loss of MLL2 in mouse embryonic stem cells (ESCs) leads to skewed differentiation, but evidence for a connection to H3K4 methylation is weak (Lubitz et al., 2007). MLL-deficient ESCs are defective in hematopoiesis (Ernst et al., 2004), but we do not know if this holds true for MLL3, MLL4, or SET1. Some studies support the role of the recently characterized DPY-30

protein as a critical regulator of MLL function (Jiang et al., 2011), although further investigation is required.

3.3.1 MLL fusions in leukemia—Leukemias harboring 11q23 translocations involving *MLL* have characteristic clinical and biological outcomes (Bernt & Armstrong, 2011). MLL-rearranged leukemias include lymphoid, myeloid, and mixed-phenotype acute leukemias phenotypes. They are found in >70% of infants with ALL and in 35–50% of infants with AML. Children with MLL-rearranged B-ALL exhibit an overall survival of ~50% versus an overall survival of >80% for children that do not harbor the translocation.

MLL rearrangements with more than 60 translocation partners have been documented. These translocation partners share no single unifying feature or functional association. The resulting MLL-fusion proteins contain the amino-terminal domain of MLL and the carboxy-terminal domain of the translocation partners. As the fusion proteins no longer contain the MLL SET domain, the oncogenic action of this chimeric protein is independent of the H3K4me3 mark. The majority of the MLL-fusion partners are part of nuclear proteins (Fig. 1.2C). Members of the so-called super elongation complex (SEC) (AF1, AF9, ENL, ELL, and AF4) are frequent fusion partners. MLL can also be fused to components of the Dot1-containing complex (DotCom) (Mohan, Lin, Guest, & Shilatifard, 2010; Smith, Lin, & Shilatifard, 2011) such as ENL (Tkachuk, Kohler, & Cleary, 1992) and AF-9 and AF-4 (Gu et al., 1992) (Fig. 1.2D). DOT1L is the catalytic component of DotCom, which facilitates di- and trimethylation of lysine 79 on histone 3 (H3K79me2, H3K79me3) (Fig. 1.2C). This histone mark is associated with actively transcribed genes and is essential for transformation by MLL-AF9 (Bernt et al., 2011; Daigle et al., 2011).

Interestingly, cross talk between the MLL-AF9 fusion protein and polycomb protein CBX8 was recently revealed in leukemia. The essential role of CBX8 in MLL-AF9-driven leukemia shows that the relationship between trithorax and polycomb group proteins is not yet fully understood (Tan, Jones, et al., 2011).

4. OTHER EPIGENETIC WRITERS, ERASERS, AND READERS

Apart from enzymes that directly add or remove epigenetic marks (writers/erasers), there are proteins that can “read” these marks. These readers can recruit other proteins that can propagate the signal and subsequent repress or activate target genes or bear themselves catalytic activity. Here, we discuss genetic and posttranslational perturbation of writers, erasers, and readers and drugs that are used against these proteins in preclinical and clinical settings in leukemia studies (Fig. 1.3A).

4.1. Arginine methyltransferases

The role of arginine methyltransferases and demethylases in tumorigenesis is poorly understood and is briefly discussed here. One methyltransferase, PRMT5, is of particular interest as this protein has been implicated in myeloproliferative neoplasms (Wysocka, Allis, & Coonrod, 2006; Zhang & Abdel-Wahab, 2012). It was shown that PRMT5 is aberrantly phosphorylated by mutant JAK2 (V617F, with increased activity) leading to decreased methylation of histones H2A and H4 and alterations in gene expression (Liu et al., 2011). Importantly, a specific inhibitor of the mutant JAK2 (Ruxolitinib) is used against MF. Inversely, the action of CCND1/CDK4 can lead to increased PRMT5 enzymatic activity in mouse lymphomas (Aggarwal et al., 2010). A putative role for PRMTs in cancers is further suggested by the fact that expression levels of both PRMT1 and 6 have been found to be elevated in different types of cancer (Yoshimatsu et al., 2011).

4.2. Lysine demethylases (KDMs)

Lysine demethylases are very important for homeostasis and cancer. There are two major families of lysine demethylases. One consists of the amine oxidases and the second of the dioxygenases. Amine oxidation by the LSD family of flavin adenine dinucleotide-dependent demethylases (Shi et al., 2004) represents a type of active demethylation reaction. KDM1A (LSD1), the first reported histone demethylase (Shi et al., 2004), catalyzes demethylation of H3K4me1 and H3K4me2 and can also demethylate H3K9me1 and H3K9me2. KDM1A has also been shown to catalyze the demethylation of nonhistone targets, such as p53 (Huang et al., 2007) as well as DNMT1 and E2F1 (Wang, Hevi, et al., 2009; Xie et al., 2011).

A second type of demethylation reaction is hydroxylation by JmjC-domain-containing proteins (Kooistra & Helin, 2012; Tsukada et al., 2006; Yamane et al., 2006). This broad family contains proteins, which catalyze demethylation of different histone and nonhistone substrates. Jumonji (Jarid2), the founding member of this family, lacks catalytic activity but plays important roles in pluripotency and development by modulating the PRC2 complex activity. JMJD3 or KDM6B, an H3K27me3 demethylase, has been reported to facilitate transcriptional initiation and elongation (Chen et al., 2012). UTX (KDM6A) and JMJD3 interact with the chromatin remodeling complex SWI/SNF (Miller, Mohn, & Weinmann, 2010), as well as MLL complexes, showing the diversity of interactions and actions of the group.

The role of lysine demethylases in tumorigenesis has been exemplified by KDM1A and KDM2B (FBXL10) (Harris et al., 2012; He, Nguyen, & Zhang, 2011; Schenk et al., 2012). In addition, mutations in the lysine demethylase *UTX*, which can remove the H3K27me3 mark, have been found in human cancers (van Haaften et al., 2009). A recent study focusing specifically on ALL reported a low frequency of *UTX* mutations (Mar et al., 2012). Most of these mutations were found in clinically defined high-risk patients suggesting possible future therapeutic or prognostic relevance (Mar et al., 2012).

4.3. Histone demethylases inhibitors (KDMi)

The inhibition of histone demethylases as anticancer treatment has great potential. However, so far, the use of these inhibitors has been restricted to preclinical studies. For example, recently, it was shown that tranilcypromine (TCP, a LSD1 inhibitor) has activity against myeloid leukemia cell lines that are driven by the MLL-AF9 oncogene (Harris et al., 2012).

So far, it has been reported that only acute promyelocytic leukemia (a subtype of AML) is sensitive to all-*trans* retinoic acid (ATRA) treatment. However more recently, *in vitro* combinatorial use of ATRA and TCP yielded promising results for other types of AML as well (Schenk et al., 2012). This further underlines the importance of combinatorial use of drugs in treatment of leukemia (Fig. 1.3B).

Moreover, it is very encouraging that the advent of new technologies including high-throughput screens and advanced crystallographic techniques are paving the way to specific drugs, which target structurally similar molecules that fulfill different functions in the cell. A recent example is the generation of the first specific inhibitor for the H3K27me3 demethylases (Kruidenier et al., 2012), which can allow selective pharmacological intervention across the Jumonji family.

4.4. Histone acetyl transferases

The family of histone acetyl transferases (HATs) consists of epigenetic modifiers that include CREB-binding protein (CBP), GCN5, and CLOCK. Mutations that inactivate the action of CBP were recently identified in ALL (Mullighan et al., 2011) and in B-cell

lymphoma (Pasqualucci et al., 2011). The MOZ (monocytic leukemia zinc-finger protein) and MORF (MOZ-related factor) HATs are important for different developmental programs and have been implicated in leukemogenesis and other tumorigenic processes. In AML, the *MOZ* gene on chromosome 8p11 is fused to the CBP gene on 16p13, producing a transcript encoding the fusion protein MOZ-CBP (Borrow et al., 1996). Interestingly, the *MORF* gene has been identified (Champagne et al., 1999) fused to CBP in AML or MDS (Kojima et al., 2003; Yang & Ullah, 2007).

4.4.1 HAT inhibitors—Three HAT inhibitors (HATi) have been described to date. Curcumin (Shehzad, Wahid, & Lee, 2010) is broad acting inhibitor that also targets p300/CBP. Garcinol (Balasubramanyam et al., 2004) and anacardic acid (Sun, Jiang, Chen, & Price, 2006) are both p300 and KAT2B inhibitors. These three inhibitors are currently in preclinical development.

4.5. Histone deacetylases

HDACs typically play a repressive role in transcription as they remove the activating acetylation marks from gene control elements. However, HDACs have also been detected at the promoters of transcribed genes (Wang, Zang, et al., 2009). There are four classes of HDACs and three of them depend on the substrate-Zn chelation in their active site. Sirtuins (Type III HDACs) are the exception to this rule as they depend on NAD⁺ for their action. No genetic perturbations affecting HDACs have been described in leukemia to date. However, differential expression of the HDACs has been associated with other types of cancer. In the absence of retinoic acid, RARα plays a suppressive role in transcription through the recruitment of corepressors such as NcoR, SMRT, Sin3a, and HDACs. The PML-RARα fusion protein is a stronger repressor than endogenous RARα (Uribealago & Di Croce, 2011), thereby warranting the use of HDACs in this scenario.

4.5.1 HDAC inhibitors—HDACs can be chemically classified as short-chain fatty acids, hydroxamic acids, cyclic peptides, and benzamide derivatives (Masetti, Serravalle, Biagi, & Pession, 2011). HDAC inhibition can lead to different outcomes, such as cell cycle arrest, differentiation, or apoptosis. The most widely used class of HDACs is the hydroxamic acids, which include trichostatin A and vorinostat (SAHA). SAHA has been approved for the treatment of several hematological malignancies, including cutaneous T-cell lymphoma (CTCL). Another hydroxamic acid, Panobinostat, is currently being subjected to trials in CML, refractory CTCL, and MMs (Wolf et al., 2012). Belinostat is another investigational HDACi and has demonstrated encouraging results in peripheral T-cell lymphoma (Copeland, Buglio, & Younes, 2010). In addition, romidepsin is a cyclic peptide (FK228) approved for CTCL. Benzamide derivatives (MGCD-0103) are a separate class of investigational drugs, in clinical development for the treatment of hematological malignancies and solid tumors. Sirtuin inhibitors have not been comprehensively studied to date. Cambinol, a sirtuin inhibitor that is structurally unrelated to other HDACi, has been shown to lead to apoptosis in BCL6-expressing Burkitt's lymphoma cells through inhibition of SIRT1 and SIRT2 (Heltweg et al., 2006). Overall, this is a very big family of inhibitors, having two members FDA approved for CTCL treatment.

4.6. Bromodomain-containing proteins

Another family of histone readers is the bromodomain (BRD)-containing protein family. The BRD recognizes acetylated residues and comprises a highly conserved, four-helix, left-twisted bundle with a characteristic hydrophobic cleft between two conserved loops. The BRD is present in the bromodomain and extra-terminal (BET) proteins as well as in members of the chromatin remodeling complexes (Snf2), the MLL complex and members of the SEC (Belkina & Denis, 2012).

Recently, BET domain-containing proteins have been found to play a key role in the development of MM (Delmore et al., 2011) mainly through the induction of the *c-Myc* gene. Another example is MLL-fusion proteins containing components of the SEC, including PAFc and pTEFb that contain BET proteins (Dawson et al., 2011). These MLL-fusion proteins can activate transcription of potent oncogenes, such as BCL2, MYC, and CDK6.

4.6.1 BRD inhibitors—Recently, James Bradner and his group modified a thienodiazepine molecule so that it inhibited the binding of BRD4 to the acetylated residues of histone H4 (Filippakopoulos et al., 2010). This so-called JQ1 inhibitor abruptly inhibits MYC expression and the MYC-associated transcriptional signatures in MM. In MLL-fusion leukemias (Dawson et al., 2011; Delmore et al., 2011; Filippakopoulos et al., 2010), inhibition of the BET proteins with a specific inhibitor (GSK1210151A (I-BET151)) lead to displacement of BRD3/4 and components of the SEC from chromatin improving the survival in mouse models of MLL-rearranged leukemia (Dawson et al., 2011). While BET proteins are involved in broad cellular processes, these two examples show that their inhibition may actually be feasible as a potential cancer therapy.

4.7. Plant homeodomain-containing proteins

The plant homeodomain (PHD) recognizes the various methylation states of lysine 4 residue on histone 3 (H3K4). In addition, affinity of the PHD for H3K9me3 has also been documented in the case of JARID1C (Iwase et al., 2007). JARID1C is a histone demethylase for H3K4me3, which suggests cross talk between different histone marks.

Translocation of PHD-containing proteins is highly prevalent in hematopoietic malignancies (Chi, Allis, & Wang, 2010). Specifically, the PHD of PHF23 and KDM5A that recognizes H3K4me3/2 has been found to be fused to the Nucleoporin 98 (NUP98) gene. NUP98 is a nuclear pore complex component. The NUP98 chimeric protein leads to aberrant transcriptional activation. The resulting fusion protein inhibits the removal of H3K4me3 and the repressive action of EZH2 complex (Wang, Song, et al., 2009).

4.8. Chromatin remodeling complexes

Intriguingly, no mutations in chromatin remodeling complexes, such as the BRG family, have been identified to date in hematopoietic malignancies (Wilson & Roberts, 2011). This probably suggests that this family has key roles in cellular physiology and mutations, even heterozygote ones, could affect key cellular processes.

5. NOVEL ASPECTS AND TECHNOLOGIES IN EPIGENETICS: IMPLICATIONS FOR LEUKEMIA

5.1. Combinatorial epigenetic marks

Recent progress suggests that the histone marks do not act alone but in highly concerted combinations. The first example came from studies on ESCs, where the so-called bivalent domains (Bernstein et al., 2006) consist of the activating mark H3K4me3 and the repressive mark H3K27me3. Genes that display these marks are poised for activation or repression and their levels of transcription are fine-tuned by the relative levels of the two marks or by other stimuli. During differentiation, these genes are either up- or downregulated leading to the subsequent removal of the respective mark.

Another example of combinatorial histone marks can be found on active genes. These genes can display the simultaneous presence of both H3K4me3 and the elongating mark H3K36me3 (Guenther, Levine, Boyer, Jaenisch, & Young, 2007). There are several other paradigms of cross talk between epigenetic marks (Zhou et al., 2011). In addition, these

marks can occur both on histone tails and the DNA itself. For example, H3K4me3 is typically associated with low levels of DNA methylation (Meissner et al., 2008; Weber et al., 2007).

It is not surprising that cancer cells have aberrations in their combinatorial histone marks. For example, Fraga et al. (2005) have reported that loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Strikingly, this loss of histone acetylation leads to a following loss of DNA methylation. This implies that physiological and cancer histone marks must be interpreted in a combinatorial mode.

One of the challenges has been to define how combinations of epigenetic marks reflect different chromatin states. One method to establish this is by studying the genome-wide localization of epigenetic marks. This has been done, for example, in human cancer cell lines (Ernst et al., 2011), *D. melanogaster* (Filion et al., 2010), and mammals (Ram et al., 2011). A recent study generated by Bernstein and colleagues described the binding of multiple chromatin modulators and transcription factors in a myeloid cell line and in ESCs. Analysis of the results revealed six classes of chromatin modules. These chromatin modules are characterized by different combinations of chromatin regulators. This study showed that although chromatin regulators might reside at different loci in the genome of different cell types, they do act on these loci in similar fashion (Ram et al., 2011).

The findings described above provide us with the tools to understand how mutations of epigenetic regulators in cancer could affect combinatorial chromatin modules. For instance, there is the fact that a lot of these factors interact with each other (such as EZH2 and DNMT (Vire et al., 2006), UTX and MLL (Issaeva et al., 2007; Lee et al., 2007)). Different models have been used to describe the functional outcome of various epigenetic states (Ernst & Kellis, 2010; Hon, Hawkins, & Ren, 2009).

5.2. Novel aspects of regulation and epigenetic factors in cancer

Recently, a novel class of RNAs, termed long noncoding RNAs (lncRNAs) was discovered. Strikingly, specific lncRNAs, such as HOTAIR, have also been found to promote cancer metastasis. The effect of HOTAIR has been reported to be through interaction with the PRC2 complex (Gupta et al., 2010). Another study by the same group showed that HOTAIR could actually interact with both PRC2 and LSD1 complexes bridging by this way H3K27 methylation with H3K4me3 demethylation leading to gene repression (Tsai et al., 2010). Another lncRNA, HOTTIP, has been found to mediate activation of the distal HOXA genes through recruitment of and MLL-containing methyltransferase complex (Wang et al., 2011).

Moreover, a number of studies displayed the importance of chromosomal interactions and the integrity of the nuclear architecture in cancer. For example, Roix, McQueen, Munson, Parada, and Misteli (2003) demonstrated that there is a correlation between the spatial proximity of two loci in normal cell development and the likelihood of translocation during carcinogenesis. Specifically, in physiological circumstances, the *MYC* gene resides in close proximity to the *IGH* and *IGL* loci. These are the exact translocation partners for Myc as found in leukemia (Chiarle et al., 2011; Klein et al., 2011). Lieberman-Aiden et al. (2009) capitalized on techniques such as HiC to map the landscape of inter- and intrachromosomal associations in myeloid leukemia and lymphoblastoid cell lines. This paved the way for new studies that assayed the differences in interchromosomal associations, the translocation landscape, and the transcriptome between normal and cancer cells. A comprehensive study by Zhang, McCord, et al. (2012) evaluated the genome-wide correlation between translocations and chromosomal interactions. This study provided further evidence for the fact that spatial proximity is positively correlated with the potential to generate chromosomal translocations. In addition, a recent study by Hakim et al. (2012) correlated

the action of AID, an enzyme that causes breaks to DNA, to the presence of translocations. This study showed that stimuli, such as DNA damage, can also affect the frequency of translocations.

The importance of nuclear architecture in the process of tumorigenicity is further underscored by the fact that lamin, a protein important for the maintenance of nuclear architecture, is also strongly associated with epigenetic regulation. Moreover, DNA methylation studies in prostate cancer showed that lamin-associated areas exhibit local hypermethylation (Berman et al., 2012). A recent study showed the extent of associations resulting from RNA polymerase activity in cancer cell lines, and the association between the respective loci and various disease states (Li et al., 2012).

Taken together, it has become clear that in order to understand cancer we will have to look at the full picture. This includes mutations, epigenetic changes, transcriptional changes, and possibly larger order chromatin interactions. Nowadays, there is no reliable epigenetic marker that can be used as a prognostic or diagnostic marker for leukemia. DNA methylation, particularly of CpG islands of DNA repair enzymes, has been shown a potential to be a useful prognostic marker in some types of cancer (Van Neste et al., 2012), but there is a long way to go before this becomes an established practice. Overview of the cancer's full properties will allow us to better estimate its potential. Ultimately, this leads to a better prognosis estimate and potentially it will allow for prediction of treatment outcome.

Acknowledgments

We thank the members of the Aifantis' laboratory for critical reading of the chapter and useful comments on the work. I. A. is a Howard Hughes Medical Institute (HHMI) Early Career Scientist and is also supported by the National Institutes of Health (RO1CA133379, RO1CA105129, R21CA141399, RO1CA149655, and RO1GM088847), the Leukemia & Lymphoma Society, the V Foundation, the American Cancer Society (RSG0806801), the Irma T. Hirsch Trust, and the Dana Foundation. P. N. is supported by a fellowship from Lady Tata Foundation for Leukemia. J. M. is financially supported by the Netherlands Organisation for Scientific Research (NWO Rubicon) and by the Dutch Cancer Society (KWF Fellowship Buit 2012–5358). T. T. is supported by the NIH (training grant T32 CA009161).

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Figure 1.1.

The role of DNA methylation in leukemia. (A) Wild-type IDH converts isocitrate to oxoglutarate. Mutations in IDH1 and 2 as found in myeloid leukemias change the activity of the enzyme. Mutant IDH converts oxoglutarate to 2-hydroxyglutarate. Oxoglutarate is a cofactor to dioxygenases like the TET proteins. TET proteins convert 5-methylcytosine to 5-hydroxymethylcytosine. This potentially leads to a demethylation of the DNA, which will permit transcription from a previously silent locus. (B) Overview of the effect of the different enzymes that regulate DNA methylation. When CpG islands are unmethylated, transcription can occur from that locus. (i) DNMT enzymes methylate CpG islands in the promoter, this leads to repression of transcription from this locus. (ii) TET proteins can

oxidize the methylcytosine to 5-hydroxymethylcytosine. (iii) The outcome of this reaction is not yet fully understood, but it is suggested that this leads to demethylation permits transcription. (See Color Insert.)

Figure 1.2.

Genetic perturbations impacting EZH2 and MLL proteins. (A) EZH2, the catalytic subunit of PRC2, represses gene activity by methylation of H3 on lysine 27. (B) Representative distribution of *EZH2* mutations reported in T-ALL, myeloid disorders (MDS, MPN, CMML, AML), and DLBCL. (C) The wild-type MLL protein is the catalytic subunit of mammalian COMPASS-like complexes which enhances gene activity through methylation of H3 on lysine 4. MLL-fusion proteins frequently associate with members of DotCom to regulate methylation of H3 on lysine 79. (D) MLL-fusion proteins typically do not involve the Set methyltransferase domain but rather the N-terminal AT hooks and CxxC domain. Frequent MLL-fusion partners include AF-9, AF-4, and ENL. (See Color Insert.)

Figure 1.3.

Major epigenetic modifiers that are genetically affected in leukemia, their associated marks and the corresponding inhibitors. (A) Major epigenetic modifiers with the corresponding inhibitors (marked with the letter i). Inhibitors that are being used for the treatment of hematopoietic malignancies are shown in red. HDAC (vorinostat and romidepsin) and DNMT inhibitors vidaza (5-azacytidine) and decitabine (5-aza-2-deoxycytidine) are currently used against MDS and CTCL correspondingly. Ruxolitinib is a JAK2 inhibitor used against myelofibrosis. HAT inhibitors, such as curcumin, have been used in clinical trials against leukemia and other hematopoietic malignancies. Other inhibitors used in the lab include histone (lysine), methyltransferase (KMTi) and demethylase (KDMi) inhibitors,

and sirtuins inhibitors (SIRTi). (B) Recently, different combinations of different epigenetic inhibitors, as well as combinations of epigenetic inhibitors with drugs inhibiting signaling transduction pathways, or chemotherapy (such as alkylating agents) are being used in clinical trials. (See Color Insert.)